

CLAIMS

1. Method for synthesis of  $\alpha$ -L-aspartyl-L-phenylalanine methyl ester by enzymatic  
5 deformylation of an *N*-formyl- $\alpha$ -L-aspartyl-L-phenylalanine compound, characterized in that *N*-formyl- $\alpha$ -L-aspartyl-L-phenylalanine or its methyl ester is treated with an enzyme having formylmethionyl peptide deformylase activity and  
10 having as a co-factor bivalent metal ions chosen from the group of group 5 to 11 metals from the periodic system of elements.
2. Method according to claim 1, characterized in that the enzyme having formylmethionyl peptide  
15 deformylase activity is an enzyme having the activity as described for EC 3.5.1.27.
3. Method according to any of claims 1 or 2, characterized in that the enzyme having formylmethionyl peptide deformylase activity  
20 contains the sequences of (i) HEXXH, (ii) EGCLS and (iii) GXGXAAXQ.
4. Method according to any of claims 1-3, characterized in that the enzyme having formylmethionyl peptide deformylase activity is  
25 obtainable from *E. coli*.
5. Method according to any of claims 1-4 , characterized in that the bivalent metal ions are manganese, iron, cobalt and nickel ions.

6. Method according to claim 5, characterized in that the bivalent metal ions are iron and/or nickel ions.
7. Method according to claim 6, characterized in that the bivalent metal ions are iron ions and all treatments with the enzyme having formylmethionyl peptide deformylase activity are carried out in the presence of a stabilisation agent.
8. Method according to claim 7, characterized in that the stabilisation agent is catalase or a trialkylphosphine compound or derivative.
9. Method according to claim 8, characterized in that the stabilisation agent is catalase.
10. Method for the preparation and recovery of  $\alpha$ -L-aspartyl-L-phenylalanine methyl ester by enzymatic deformylation of an *N*-formyl- $\alpha$ -L-aspartyl-L-phenylalanine compound, characterized in that either (i) a mixture of *N*-formyl- $\alpha$ - and *N*-formyl- $\beta$ -L-aspartyl-L-phenylalanine or (ii) a mixture of *N*-formyl- $\alpha$ - and *N*-formyl- $\beta$ -L-aspartyl-L-phenylalanine methyl ester is treated with an enzyme having formylmethionyl peptide deformylase activity and having as a co-factor bivalent metal ions chosen from the group of group 5 to 11 metals from the periodic system of elements, with the formation of  $\alpha$ -L-aspartyl-L-phenylalanine or of its methyl ester, respectively, whereby in case  $\alpha$ -L-aspartyl-L-phenylalanine is formed in the deformylation step a subsequent methylation

step of the phenylalanine carboxylic acid group is carried out, and the  $\alpha$ -L-aspartyl-L-phenylalanine methyl ester is recovered.

11. Method according to claim 10, characterized in  
5 that the enzyme having formylmethionyl peptide deformylase activity is an enzyme having the activity as described for EC 3.5.1.27.
12. Method according to any of claims 10 or 11,  
10 characterized in that the enzyme having formylmethionyl peptide deformylase activity contains the sequences of (i) HEXXH, (ii) EGCLS and (iii) GXGXAAXQ.
13. Method according to any of claims 10-12 ,  
15 characterized in that the enzyme having formylmethionyl peptide deformylase activity is obtainable from *E. coli*.
14. Method according to any of claims 10-13,  
characterized in that the bivalent metal ions are manganese, iron, cobalt and nickel ions.
- 20 15. Method according to claim 14, characterized in that the bivalent metal ions are iron and/or nickel ions.
16. Method according to claim 15, characterized in  
25 that the bivalent metal ions are iron ions and all the treatments with the enzyme having formylmethionyl peptide deformylase activity are carried out in the presence of a stabilisation agent.

17. Method according to claim 16, characterized in that the stabilisation agent is catalase or a trialkylphosphine compound or derivative.
18. Method according to claim 17, characterized in that the stabilisation agent is catalase.
19. Method for synthesis of  $\alpha$ -L-aspartyl-L-phenylalanine methyl ester by enzymatic deformylation of an *N*-formyl- $\alpha$ -L-aspartyl-L-phenylalanine compound, characterized in that *N*-formyl-L-aspartic acid is coupled enzymatically, using thermolysin as the coupling enzyme, with L- or D,L-phenylalanine methyl ester, and that simultaneously, and in the same reaction vessel, the *N*-formyl- $\alpha$ -L-aspartyl-L-phenylalanine methyl ester formed by the coupling reaction is deformylated by an enzyme having formylmethionyl peptide deformylase activity and having as a co-factor bivalent metal ions chosen from the group of group 5 to 11 metals from the periodic system of elements and being present in the reaction system for the enzymatic coupling reaction.
20. Method according to claim 19, characterized in that, the  $\alpha$ -APM so formed is recovered after the reaction has proceeded till a conversion of more than 40%.
21. Method according to any of claims 19 or 20, characterized in that the enzyme having formylmethionyl peptide deformylase activity is an enzyme having the activity as described for EC 3.5.1.27.

22. Method according to any of claims 19-21,  
characterized in that the enzyme having  
formylmethionyl peptide deformylase activity  
contains the sequences of (i) HEXXH, (ii) EGCLS  
5 and (iii) GXGXAAXQ.
23. Method according to any of claims 19-22,  
characterized in that the enzyme having  
formylmethionyl peptide deformylase activity has  
a deformylating activity towards (oligo)peptides  
10 with *N*-formylmethionine at their *N*-terminus,  
which is at least 10x higher, preferably at least  
100x higher, and most preferred at least 200x  
higher than its deformylating activity towards *N*-  
formyl methionine.
- 15 24. Method according to any of claims 19-23,  
characterized in that the enzyme having  
formylmethionyl peptide deformylase activity is  
obtainable from *E. coli*.
25. Method according to any of claims 19-24,  
20 characterized in that the bivalent metal ions are  
manganese, iron, cobalt and nickel ions.
26. Method according to claim 25, characterized in  
that the bivalent metal ions are iron and/or  
nickel ions.
- 25 27. Method according to claim 26, characterized in  
that the bivalent metal ions are iron ions and  
all treatments with the enzyme having  
formylmethionyl peptide deformylase activity are  
carried out in the presence of a stabilisation  
30 agent.

28. Method according to claim 27, characterized in that the stabilisation agent is catalase or a trialkylphosphine compound or derivative.
29. Method according to claim 28, characterized in that the stabilisation agent is catalase.
- 5 30. Method for the synthesis of di- or oligopeptides or derivatives thereof from two starting materials, the first of which is an N-formyl protected amino acid which is capable of undergoing an enzymatic coupling reaction with a second amino acid or derivative thereof, or with a di- or oligo-peptide or derivative thereof, thereby yielding an N-formyl protected reaction compound, wherein the N-formyl protecting group of the first starting material is retained during the enzymatic coupling reaction with the second starting material, whereby said protecting group is cleaved off enzymatically, using an enzyme having formylmethionyl peptide deformylase activity and having as a co-factor bivalent metal ions chosen from the group of elements, from 20 metals from the periodic system of elements, from the reaction compound at a substantially higher, i.e. at least 10x higher, rate than from the first starting material, and wherein two enzymes are involved simultaneously for the enzymatic coupling reaction between the starting materials and the enzymatic deformylation of the reaction 25 compound.